VOL. VIII, No. 10 DECEMBER, 1947

BORDEN'S REVIEW

of

NUTRITION RESEARCH

NUTRITIONAL FACTORS IN LIVER DISEASE

BORDEN'S REVIEW
of
NUTRITION RESEARCH

Reviews and summaries of research information concerning nutrition and foods. Published monthly, except July and August, by THE BORDEN COMPANY; officers of the Company: Theodore G. Montague, president; E. L. Noetzel, treasurer; T. D. Waibel, secretary. Editorial office: 350 Madison Avenue, New York 17, N. Y.

NUTRITIONAL FACTORS IN LIVER DISEASE

By RUTH WOODS

During the past two decades research investigations have culminated in the significant discovery that specific factors (the so-called lipotropic substances) in the diet have an important effect upon the control of fat deposition in the liver.* That this finding may be of more than experimental interest becomes apparent when it is recognized that a condition of fatty, enlarged liver is sometimes a precursor of liver cirrhosis and other liver diseases in man. The study of lipotropic factors, which had its origin in physiological researches, has been augmented by findings concerning the influence of other dietary factors on the metabolic functioning of the liver. These concepts have revolutionized the rationale of early methods of therapy, particularly those which advocated the use of extremely restricted diets. Today, nutrition therapy in liver disease is considered an essential adjunct to routine medical and surgical procedures. The essence of the modern therapeutic diet in liver disease is the provision of liberal amounts of calories, protein and carbohydrate, reduced amounts of fat, supplementation with vitamins (especially those of the B complex) and with the lipotropic factors such as choline, its precursors and related compounds.

Lipotropic Factors: Mechanisms of Activity:

The lipotropic factors, choline, methionine, lipocaic and inositol, constitute a major group in the list of modern therapeutic agents in liver disease. Their action is directed chiefly against the deposition of excess fat in the liver, but indirectly they have been found to exert some influence on other forms of liver disease as well. While the evidence for the existence of specific lipotropic factors is convincing enough, considerable speculation still exists as to the manner whereby these factors function. Only in the case of the proteins is the evidence relatively conclusive. The mechanism of choline activity has been convincingly demonstrated, but may warrant reinterpretation in the light of changing concepts of fat metabolism. The mechanism of the action of the pancreatic factors, lipocaic and the anti-fatty liver factor of Chaikoff *et al* has been hinted at in recent investigations, but must necessarily await chemical identification of these factors.

Choline: Choline and certain of its related compounds are thought to exert their lipotropic effect by stimulating the rate of turnover of phospholipid formation in the liver—specifically by being incorporated into newly

*cf. *Borden's Review of Nutrition Research,* November, 1947—"Nutritional Significance of Lipotropic Factors."

formed lecithin molecules. This explanation is based on the current belief that phospholipid formation is an essential prerequisite to fat transport and utilization.

That choline actually does stimulate the rate of phospholipid turnover has been demonstrated unequivocally through the use of the "tracer" technique. The first experiment of this kind was that of Welch (1) more than ten years ago. Isotopes were not then available for biological research, but Welch devised an ingenious means of "labelling" choline so that its course in the body could be determined. He introduced arsenic into the choline molecule, thereby creating an easily detectable form of choline. Arsenocholine was fed to animals and found to behave like choline and to possess similar lipotropic power. A search for the ingested arsenocholine disclosed that most of it had found its way into the phospholipids. Arsenocholine does not function as a methyl donor. This suggests that its lipotropic activity is dependent upon the intact molecule and implies that choline, likewise, is utilized as the intact unit with respect to its lipotropic behavior.

With the availability of radioactive phosphorus (2) and of heavy nitrogen (3), complete confirmation was obtained of the fact that choline and related compounds accelerate the rate of phospholipid formation.

If the phospholipid theory of fat transport is valid, the mechanism of choline activity is clear-cut and logical. In a review on phospholipid metabolism in 1941, however, Sinclair (4) cited important studies by Hevesy and Hahn and others which seem to cast serious doubt on this generally accepted theory. He concludes that it may not be justifiable to interpret the comparatively rapid rate of turnover of phospholipids in the liver (and elsewhere) as evidence of their active participation in fatty acid metabolism. Whatever the outcome of this question may be, the significant fact nevertheless remains that a decided increase in phospholipid turnover occurs when choline is provided in the diet.

Proteins: The lipotropic activity of the proteins is due primarily to their methionine content and to their methionine-cystine ratios. A possible secondary factor, as yet unidentified, may be tyrosine. The lipotropic activity of methionine, in turn, has already been explained on the basis of its contributing methyl groups for the synthesis of choline. It is important to realize that while labile methyl groups are essential for the synthesis of choline, they are not essential to the phenomenon of lipotropism. A lipotropic compound may or may not contain methyl groups—the lipotropic effect is dependent rather upon the entire structural unit of the compound. Beveridge *et al* (5) have recently concluded that the lipotropic activity of a protein is determined not only by its methionine and cystine contents, but

also by the nature and quantity of the *sulfur-free* essential amino acids in the protein. These amino acids do not act directly, but through their influence on growth and maintenance they influence the formation of new tissue, thus modifying the amount of methionine left available for lipotropic action.

Pancreas Factors: In very recent work by Chaikoff, Entenman and Montgomery (6, 7, 8) on the anti-fatty liver factor of pancreas a number of interesting facts have been brought to light. Of particular significance is their finding that both the totally depancreatized dog and the animal deprived of pancreatic juice only (by tieing off the pancreatic ducts) suffer from a deficiency of plasma choline in the circulating blood. The fall in plasma choline is associated with the development of fatty livers. Recent studies by this group had already established the fact that more than 95 per cent of the normal blood phospholipids is of the choline-containing type, representing a concentration of virtually all of the choline found in plasma. These facts strongly suggest that the plasma phospholipids are intimately connected with the physiological activity of *circulating* choline. This introduces a role of phospholipids not previously stressed. As the authors point out:

> "Although it has been widely accepted that phospholipids serve to transport fat, their role as vehicles for the storage and transport of choline has hitherto not been stressed."

The mechanism whereby the pancreatic juice influences the level of plasma choline is not yet apparent. Since extracts of pancreatic juice or of pancreatic tissue increase the blood choline *independently of the choline content of the diet,* it is assumed that the extra choline is mobilized from the tissues or synthesized from its precursors. In a subsequent report (7) these investigators found that the administration of free methionine prevented the fatty livers in dogs deprived of pancreas and pancreatic juice. This indicated that the synthesis of choline from *free* methionine is not interfered with in depancreatized animals. Thus the most plausible explanation of the action of the anti-fatty liver extract of pancreas appears to be that it makes available for lipotropic purposes the *bound* methionine contained in dietary proteins. This suggests further a possible enzymatic nature of the unidentified pancreas factor.

In April of this year, Chaikoff's group (7) presented further evidence in support of their hypothesis that the anti-fatty liver factor of pancreas and pancreatic juice is similar to a proteolytic enzyme which is necessary for ingested proteins to exert their full lipotropic effect. In support of this theory they present evidence that while purified casein fails to prevent fatty livers in depancreatized dogs, hydrolyzed casein is completely lipotropic. In other words, before the methionine of casein can be utilized in the body, casein

must first be digested (presumably by enzymatic action of the pancreas). This explains the apparent discrepancy in work with depancreatized dogs which were found to develop fatty livers on a high protein diet—a regimen which normally should have been protective against the liver disturbance.

Support of the theory of the proteolytic nature of pancreas extracts was found in studies reported last year from Best's (9) laboratory in which Palmer had presented preliminary evidence that the lipotropically active fraction of pancreatic juice contains three proteolytic enzymes, two of which were identified as trypsin and chymotrypsin.

The question of whether these two enzymes are identical with the anti-fatty liver factor of pancreas has not yet been resolved. However, it is interesting to note the return, as a result of modern investigations, to the theory concerning the importance of digestive enzymes in pancreas suggested by the very early workers on diabetic dogs in Macleod's laboratory.

Experimental Liver Cirrhosis:

During the course of studies begun in 1935 on pyridoxine deficiency in rats, György and Goldblatt (10) reported in 1939 the unexpected death of many of their animals on an experimental pyridoxine-deficient diet adequately supplemented with thiamine and riboflavin. The stricken animals, strangely enough, were free from specific symptoms of acrodynia (the characteristic manifestation of pyridoxine deficiency) as well as from other diseases which sometimes complicate the picture of experimental vitamin B_6 deficiency. The cause of death among these animals had remained an unsolved mystery for about two years until the casual observation of yellowish red patches in several lobes of the liver of one animal suggested the possibility that the cause of death might be traced to injury to the liver. From that point on, all of the animals on the pyridoxine-deficient basal diet dying of unknown causes were examined microscopically for evidence of liver damage. During the year and one-half period which followed, forty-eight animals were found with markedly pathological changes in the liver.

Infection, or the presence of toxins, was ruled out after careful inquiry and study, leaving only nutritional factors as the apparent cause of the liver injury. Since the liver damage appeared even when pyridoxine supplements were added to the diet, but not when yeast or yeast extract was added, it seemed likely that some nutritional factor in yeast (other than pyridoxine, riboflavin or thiamine) was effective in protecting the animals and that the absence of this factor or factors in the synthetic experimental diet was the predisposing cause of the liver damage.

The liver damage was found to resemble that induced by various kinds of poisons such as carbon tetrachloride or chloroform. The changes were characterized by local death of the liver tissue, hemorrhage, fatty degeneration and the replacement of normal liver tissue with inert fibrous tissue suggestive of cirrhosis.

The following year, Rich and Hamilton (11) reported the production by dietary means of a cirrhosis of the liver in rabbits which closely resembled a characteristic type in man. Here again evidence indicated that the lesions were due to the lack of some substance in yeast, but not specifically to the absence of thiamine, riboflavin, pyridoxine or nicotinic acid. The pathological changes were definitely cirrhotic in nature as evidenced by the formation of characteristic scar tissue. The fat content of the livers varied considerably, in general becoming proportionately less as the scarring process advanced. Since it was noted that the diet was deficient in choline, it is possible that this substance was at least in part involved in the production of the liver lesions.

The early observations of György and Goldblatt and of Rich and Hamilton represented some of the first evidences that dietary inadequacy may induce not only fatty livers, but also a degenerative disease closely resembling certain phases of human liver cirrhosis.

Relation of Lipotropic Factors to Cirrhosis: As studies on the experimental production of liver cirrhosis by dietary means expanded, it soon became apparent that a deficiency of the same factors involved in the production of fatty livers was, under long-term conditions, also responsible for the development of cirrhosis.

As early as 1938, Connor and Chaikoff (12) reported the production of liver cirrhosis in dogs by means of a high fat diet and alcohol. Some years later they reported the induction of cirrhosis on a high fat diet alone, without alcohol (13). In the meantime, the preliminary work of György and Goldblatt (10) and of Rich and Hamilton (11) had appeared further indicating the possibility of a dietary cause of cirrhosis. By 1941, four independent groups of workers announced almost simultaneously the fact that cirrhosis could be induced by a low protein, high fat diet and that it could be decreased in severity or even prevented by supplementation with the lipotropic factors, choline, betaine, methionine or higher levels of casein.

György and Goldblatt (14) found they could regularly produce the liver injury observed in their earlier studies by means of a diet containing half or less the amount of casein used in normal diets. They reported that supplements of choline in the amount of 10 to 20 grams per day lessened the

incidence and severity of the injury but did not completely prevent it. In a later study (15) they found that extra casein or a combination of cystine and choline was more nearly preventive of the injury. Cystine alone, however, exerted a definite deleterious effect on the liver. At about the same time, Webster (16) also reported successful induction of cirrhosis in rats on a low protein diet containing about 38 per cent of fat. Increasing the protein (casein) from 8 to 18 per cent, decreasing the fat and adding betaine was found to prevent the disease. Like György *et al,* Webster found that cystine alone, or cholesterol, aggravated the condition. Whole yeast or molasses were found to provide other unidentified preventive factors.

Coincident with these reports came the work of Blumberg and McCollum (17) demonstrating the protective action of choline on experimentally induced cirrhosis. These workers had noted the occurrence of liver cirrhosis in several animals used in earlier studies concerned with the feeding of wheat germ oil. The diets in these earlier studies had been high in fat (wheat germ oil) and low in protein and were used as a pattern for later diets designed to produce cirrhosis. Large amounts of choline, 40 to 60 grams per day, were found to prevent the development of cirrhosis on these diets. Methionine supplements in the amount of 25 mg. per day retarded cirrhosis but did not prevent it, while the addition of cystine seemed to be without any effect.

Additional information on this subject appeared with the publication of a paper by Lillie, Daft and Sebrell (18) in which it was shown that alcohol aggravated the severity of cirrhosis produced by a low protein, low methionine and cystine diet. In a subsequent experiment these workers (19) reported the consistent production of cirrhosis on a low protein, *low fat* diet with added cystine and indicated its apparent prevention by choline (20 mg. per day), by methionine (a level of 0.7 per cent of the diet) or by casein (raised from 4 to 30 per cent of the diet), either singly or in combination. Animals on the experimental diet all developed liver pathologies whether or not alcohol was substituted for drinking water, thereby indicating that diet and not alcohol was the essential factor in producing the liver damage.

Interpretation of Findings: From the experimental evidence available it seems quite apparent that the lipotropic factors are of primary importance in the prevention of experimental liver cirrhosis. Some confusion still exists, however, concerning the significance and role of the individual factors. As has been indicated earlier in this Review, the mechanisms of lipotropic activity are believed to be tied up with the functioning of the intact choline molecule. Proteins are believed to be lipotropic largely because of their methionine content. The lipotropic action of methionine, in turn, is ex-

plained on the basis of its available methyl groups which permit the synthesis in the body of *choline* from suitable, non-methylated precursors. The interpretation of the behavior of these compounds with respect to their cirrhosis-preventing ability, however, is still somewhat obscure. György (20) in his earlier studies suggested that methionine is superior to choline in the prevention of cirrhosis and pointed out further that choline plus cystine was more effective than choline alone. (Cystine alone, however, has been found to aggravate cirrhosis as it does the formation of fatty livers.) György speculated as to the possibility of the choline-cystine combination giving rise to methionine, thereby explaining its cirrhosis-preventing action. More recently, however, general opinion seems to indicate a combination of a high protein, high carbohydrate diet supplemented with choline and other B complex vitamins, rather than any one component, as the most effective means of preventing and treating liver damage (21, 22).

Modern Concepts in Liver Physiology:

The experimental production and control of fatty livers and ultimately of liver necrosis and cirrhosis by dietary means represents one of the major advances of modern times in understanding and treating diseases of the liver. The newer knowledge of the physiology of the liver which has developed progressively since the 1920's has been another outstanding factor in the interpretation of liver pathologies and in determining the course of therapy. Results gained from these two fundamental approaches have served as a basis for the evolution of present day treatment of liver disease largely along nutritional lines (23). In addition to the more recent findings concerned with the activity of the lipotropic factors, there have been significant advances in evaluating the role of carbohydrates, proteins and vitamins in the metabolism of the liver. Inasmuch as interference with normal metabolic activities is perhaps the most serious manifestation of liver disease, the importance of these findings can scarcely be overemphasized.

Role of Various Nutrients in Liver Metabolism: *(Carbohydrate)* The liver serves as one of the main storehouses of carbohydrate. Carbohydrate stores in the liver, in the form of glycogen, are readily mobile. Thus the liver stores glycogen when there is an excess of blood sugar following meals and, conversely, when the blood sugar is low the liver reconverts glycogen to glucose. An adequate store of glycogen in the liver is necessary to prevent hypoglycemia (low blood sugar) and for the detoxification of various drugs, poisons (phenol derivatives, p-aminobenzoic acid, sulfanilamide, chloroform) and bacterial toxins (24, 25, 26, 27).

(Protein): The amino acids, products of food protein digestion, are carried to the liver where they are fabricated into essential new tissue proteins, particularly the blood plasma proteins, albumin, globulin, fibrinogen and prothrombin. The plasma proteins have many important functions, including the maintenance of a dynamic equilibrium with tissue protein stores, the carrying of immunizing antibodies and the regulation of the blood-clotting mechanism. The inability of the liver to manufacture sufficient amounts of these proteins will, therefore, precipitate many physiological disturbances. An adequate store of liver protein, like glycogen, protects the liver from injury by chloroform, carbon tetrachloride, selenium, arsenic and other poisons. The mechanisms involved are not similar to those occurring in detoxification by glycogen and have not been fully explained as yet. Prolonged fasting decreases the protein content of the liver and leads to decreased levels of serum albumin in the blood and to abnormal deposition of fat in the liver.

(Fat): Fat is not stored in the liver. It is, however, constantly being mobilized to the liver, altered in structure and moved out. Ingestion of abnormally high fat diets, presence of certain toxins, removal of pancreas, starvation, ingestion of excessive amounts of alcohol and deficiency of lipotropic compounds are some factors involved in the accumulation of excess liver fat. A liver containing increased amounts of fat is particularly susceptible to hepatic toxins even if the glycogen stores are high. Fatty livers frequently precede cirrhosis in experimental animals and may sometimes do so in man, as well.

(Vitamins): Inadequate absorption, metabolism and storage of the vitamins is a common result of liver disease. Interference with the normal supply of bile salts (manufactured in the liver) prevents adequate absorption of the fat soluble vitamins. In addition the damaged liver is unable to store those vitamins which are absorbed. Thus deficient dark-adaption (vitamin A deficiency), osteoporosis (vitamin D deficiency), prolonged blood-clotting time (vitamin K deficiency) have all been reported as common complications of chronic liver disease. This necessitates the provision of extra supplies of these vitamins by parenteral administration or, when available, in water-soluble form. The requirements of the B vitamins which are stored by the normal liver are likewise heightened in liver disease. These factors are intimately involved in the oxidation of carbohydrate, in the elaboration of enzyme systems, in the synthesis of fat from carbohydrate and protein as well as in the development of the characteristic symptoms of pellagra, neuritis and ariboflavinosis. Because the damaged liver not only interferes with absorption of the vitamins, but may also fail to store or even metabolize

them, supplementation with vitamins may not always correct the effects of their deficiency unless excessive amounts are provided.

Conclusion:

Prior to the understanding of the significance of the various nutrients in the economy of the liver, treatment of liver disease was characterized by the provision of diets low in all constituents to "spare" the diseased liver as much as possible. In the light of present knowledge of liver physiology it can readily be seen that this approach was not only ineffectual but actually harmful. The provision of liberal amounts of carbohydrate and of protein has been demonstrated to be of positive value in supportive and protective therapy of the liver. The administration of vitamins is apparently beneficial, but the evidence for their efficacy is as yet insufficient. The lipotropic factors have proved their value beyond doubt in experimentally produced liver disease. Considerable clinical evidence is needed before their value in human liver disease can be conclusively established. At the present time a small but growing mass of clinical data has accumulated on the efficacy of treating chronic liver disease by supportive nutrition therapy based on the newer concepts of liver physiology and on the findings of animal investigations based on experimentally induced liver disease. These reports are preliminary, but nevertheless they indicate a hopeful outlook for the modern nutrition therapy of liver disease.

Note: A review of the clinical studies employing nutrition therapy (protein, carbohydrate, vitamins and lipotropic factors) in the treatment of human liver disease will appear in a future issue of *Borden's Review of Nutrition Research.*

REFERENCES CITED

1. WELCH, A. D. Utilization of the arsenic analogue of chloride in the biosynthesis of phospholipid. *Proc. Soc. Exp. Biol. Med. 35,* 107 (1936).

2. PERLMAN, I. and I. L. Chaikoff. Radioactive phosphorus as an indicator of phospholipid metabolism. V. On the mechanism of the action of choline upon the liver of the fat-fed rat. *J. Biol. Chem. 127,* 211 (1939).

3. STETTEN, DeW., Jr. Biological relationship of choline, ethanolamine, and related compounds. *J. Biol. Chem. 138,* 437 (1941).

4. SINCLAIR, R. G. The anabolism and function of the phospholipids. *Biol. Symp. 5,* 82 (1941).

5. BEVERIDGE, J. M., C. C. Lucas and M. K. O'Grady. The effect of dietary proteins and amino acids on liver fat. *J. Biol. Chem. 160,* 505 (1945).

6. CHAIKOFF, I. L., C. Entenman and M. L. Montgomery. The mechanism of action of the antifatty liver factor of the pancreas. I. Its relation to plasma choline. *J. Biol. Chem. 160,* 387 (1945).

7. CHAIKOFF, I. L., C. Entenman and M. L. Montgomery. The mechanism of action of the antifatty liver factor of the pancreas. II. Free methionine prevents fatty livers in completely depancreatized dogs maintained with insulin and fed a lean meat diet. *J. Biol. Chem. 160,* 489 (1945).

8. CHAIKOFF, I. L., C. Entenman and M. L. Montgomery. The mechanism of action of the antifatty liver factor of the pancreas. III. A comparison of hydrolyzed and unhydrolyzed casein in the prevention of fatty livers of the completely depancreatized dog maintained with insulin. *J. Biol. Chem. 168,* 177 (1947).

9. BEST, C. H. Pancreatic enzymes and liver fat. *Science 103,* 207 (1946).

10. GYÖRGY, P. and H. Goldblatt. Hepatic injury on a nutritional basis in rats. *J. Expt. Med. 70,* 185 (1939).

11. RICH, A. R. and J. D. Hamilton. Experimental production of cirrhosis of liver by means of deficient diets. *Bull. Johns Hopkins Hosp. 66,* 185 (1940).

12. CONNOR, C. L. and I. L. Chaikoff. Production of cirrhosis in fatty livers with alcohol. *Proc. Soc. Exp. Biol. Med. 39,* 356 (1938).

13. CHAIKOFF, I. L. and C. L. Connor. Production of cirrhosis of liver of normal dog by high fat diet. *Proc. Soc. Exp. Biol. Med. 43,* 638 (1940).

14. GYÖRGY, P. and H. Goldblatt. Experimental production of dietary liver injury (cirrhosis, necrosis) in rats. *Proc. Soc. Exp. Biol. Med. 46,* 492 (1941).

15. GYÖRGY, P., E. C. Poling and H. Goldblatt. Necrosis, cirrhosis and cancer of liver in rats fed a diet containing dimethylaminoazobenzene. *Proc. Soc. Exp. Biol. Med. 47,* 41 (1941).

16. WEBSTER, G. Dietary liver disease in rats. *J. Clin. Invest. 20,* 440 (1941).

17. BLUMBERG, H. and E. V. McCollum. Prevention by choline of liver cirrhosis in rats on high fat, low protein diets. *Sci. 93,* 598 (1941).

18. LILLIE, R. D., F. S. Daft and W. H. Sebrell. Cirrhosis of the liver in rats on a deficient diet and the effect of alcohol. *U. S. Public Health Rpt. 56,* 1255 (1941).

19. DAFT, F. S., W. H. Sebrell and R. D. Lillie. Production and apparent prevention of a dietary liver cirrhosis in rats. *Proc. Soc. Exp. Biol. Med. 48,* 228 (1941).

20. GYÖRGY, P. Experimental hepatic injury. *Am. J. Clin. Path. 14,* 67 (1944).

21. PATEK, A. J., Jr. and J. Post. Treatment of cirrhosis of the liver by a nutritious diet and supplements rich in vitamin B complex. *J. Clin. Invest. 20,* 481 (1941).

22. LEVINE, R. The physiological basis for the diet therapy of liver disease. *J. Am. Diet. Assoc. 23,* 485 (1947).

23. WEIR, J. F. Modern physiologic concepts: Their application in treatment of disease of the liver. *J. Am. Med. Assoc. 134,* 579 (1947).

24. DAVIS, N. C. and G. H. Whipple. The influence of fasting and various diets on the liver injury effected by chloroform anesthesia. *Arch. Int. Med. 23,* 612 (1919).

25. BOLLMAN, J. L. Some experimental observations pertinent to the treatment of hepatic disease. *Ann. Int. Med. 12,* 1 (1938).

26. OPIE, E. L. and L. B. Alford. The influence of diet upon necrosis caused by hepatic and renal poisons. I. Diet and the hepatic lesions of chloroform, phosphorus or alcohol. *J. Exp. Med. 21,* 1 (1915).

27. ALTHAUSEN, T. L. Dextrose therapy in diseases of the liver. *J. Am. Med. Assoc. 100,* 1163 (1933).

NEWS DIGEST

CARBOHYDRATE CALORIES — When free choice of foods is permitted, the appetites of experimental animals are generally responsive to nutritional need. Thus a complete protein is selected over one lacking in essential amino acid; a salt mixture containing calcium is selected by animals whose parathyroids have been removed, and extremely small concentrations of sodium chloride can be detected by rats whose adrenals have been removed. While it has long been known that a drop in environmental temperature stimulates an increase in the consumption of energy-yielding food, a recent study has shown that the preferred source of calories changes when the temperature is altered; whereas at room temperature mice consumed a food rich in fat, at 10 to 14 C. not only was the energy intake greater but there was a shift to a diet rich in carbohydrate. Increase to 29 to 33 C. in the environmental temperature caused a drop in the total calories consumed and a shift away from the carbohydrate food. A similar preferential use of carbohydrate was shown after the administration of thyrotoxin. These observations support the well-known concept of the preference for carbohydrate in muscle activity which frequently is confused with the demand for fat because of the satiety value of the latter. *J. Am. Med. Assoc. 135,* 718 (1947).

ANTI-FOLIC ACID — Early this year came the first report of an antimetabolite of folic acid, the only known member of the vitamin B complex for which a structurally related antagonist had not yet been reported. The anti-folic acid is *methylfolic acid* and is reported to be an effective displacer of folic acid using the microorganism *S. faecalis* R as a means of testing for folic acid activity. *Arch. Biochem. 12,* 318 (1947).

COMPARATIVE VALUE OF DIETARY PROTEINS — Two groups of children in separate orphanages were selected for a recently conducted nutrition study designed to compare the effects of two different types of diets. Both diets were planned to meet *quantitatively* the nutrition standards set by the National Research Council. The diets differed essentially in the nature but not in the amounts of the protein, one containing protein of animal origin and the other containing protein largely of vegetable origin. All of the children were considerably undernourished when the investigation began.

The study included more than 100 children and lasted for fourteen months.

The children in Institution I received meat or fish only twice weekly. Those under 10 years of age received 75 grams of meat and liver and 75 grams of poultry or fish; those ten years of age or over received 100 grams of each. In addition, legumes — macaroni, peanuts, or peanut butter were served to this group eight times weekly. The plan for Institution II provided for ten weekly servings of meat, poultry and fish. The younger children received 675 grams of meat and liver and 75 grams of poultry or fish; the older children received 900 grams of the former and 100 grams of the latter. The quantity of all other foods offered in both institutions was made equivalent for control purposes. Practical considerations prevented the master plans from being carried out to the letter; but the average diets for both institutions were comparable, except that the diet for Institution II was lower in caloric value and vitamin A intake.

All children were given thorough medical examinations — at the beginning, at eight months, and at fourteen months, which marked the conclusion of the experimental period. Scrutiny was focused upon epithelial cell integrity, the thyroid, the skeletal system, weight, the hemoglobin level and red cell count, blood plasma vitamin A, serum protein fractions, blood phosphatase levels, urinary excretion, the reflexes, tendency to fatigue, and dark adaptation. No person associated with making the medical examinations was informed of the dietary regimen; thus the possibility of subjective error was obviated.

The data accumulated on final examination revealed that the children of both institutions had improved markedly in physical well-being. But more salient was the fact that the children in Institution I (on meat or fish only twice weekly) failed to surpass the children in Institution II in any of the medical tests. The children receiving the diet which contained ample protein of high biologic value excelled or equaled the others on all counts. The authors evaluate their study in the following words: "The findings demonstrate the desirability of considering more than the numerical results of a calculation from tables of averages in making dietary recommendations for growing children." *J. Am. Diet. Assoc. 23,* 488, 588, 677 (1947).

Printed in U.S.A.